Molecular Typing of Staphylococcus Using Automated rep-PCR

S. Cook¹ and D.S. Shapiro¹,²
¹Molecular Diagnostics and Microbiology Laboratories, Department of Laboratory Medicine, Lahey Clinic, Burlington, MA
²Department of Medicine, Boston University School of Medicine

As illustrated in Figure 1: rep-PCR shows the ability to distinguish S. aureus isolates at the clonal level, indicating that it can be a useful tool for epidemiologists and hospital infection control teams.

**Background:**
Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen often associated with nosocomial and community-acquired infections.¹⁻³ Nosocomial outbreaks have a tremendous impact on the morbidity, mortality and cost associated with hospital-acquired infections. An estimated two million patients develop nosocomial infections in the United States annually.¹ The increase in the occurrence of MRSA infections demands a typing system that is quick, dependable, easy, discriminates readily between different strains, and is reproducible. The ability to quickly identify the source of an infection could have a dramatic cost savings for the healthcare facility;⁴ however, discriminating between bacterial isolates at the clonal level in a timely manner is challenging. Several molecular typing methods are used to determine possible sources of infections caused by S. aureus, with pulse-field gel electrophoresis (PFGE) being the most commonly used method. PFGE is a powerful method to discriminate between different strains of MRSA. However, the method is difficult, laborious, and does not reliably produce stable banding patterns for MRSA.⁴ Another method, repetitive sequence-based PCR (rep-PCR), has shown promise as a useful tool. Our nine-month study reports on the performance and utility of the DiversiLab System (Spectral Genomics, Inc.) in a clinical laboratory setting.

**Methods:**
A total of 335 clinical MRSA isolates obtained from 2004 to 2005 were tested. 120 clinical samples were tested retrospectively and 215 samples have been tested prospectively. Additionally, 20 samples were sent out to a reference laboratory for PFGE for comparison and 12 archived samples from 2001-2003 were retrieved from storage for database comparison.

The isolates were cultured and DNA was extracted using the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). The DiversiLab Staphylococcus Kit was used for rep-PCR amplification of non-coding intergenic repetitive elements in the genomic DNA. Amplicon detection and data analysis were performed using the DiversiLab System, consisting of the Agilent 2100 bioanalyzer and the DiversiLab web-based software.

**Results:**
Each run, performed on a single chip, consisted of 1 positive control, 1 negative control, 1 ladder control and 10 samples. Rep-PCR fingerprints could be obtained from all samples tested. 15/335 (4.5%) samples required repeat analysis because of low signal strength during detection, but upon repeat analysis a fingerprint was generated.

There were 257 MRSA isolates collected in 2004. A total of 120 (47%) of these samples were tested in our study. 95/120 (79%) of the samples from 2004 that were tested retrospectively demonstrated ≥ 95% similarity to each other.

A total of 323 isolates have been collected to date for the year 2005. A total of 215 (67%) of the samples were tested in our study. 178/215 (83%) of the samples from 2005 demonstrated ≥ 95% similarity to each other.

When samples from all years were combined into a database, a total of 347 samples were fingerprinted. Of these, 275 (79%) demonstrated ≥ 95% similarity to each other.

The 20 samples tested by rep-PCR and PFGE showed good correlation 18/20 (90%) and directly matched the results obtained from a reference laboratory that performed PFGE. The 2 samples that did not match were determined to be closely related by rep-PCR vs. possibly Related by PFGE.

Finally, the 14 archived isolates demonstrated different patterns in different years. The greatest similarity in the patterns occurred in isolates that were temporally close to each other. The 2001 isolates showed greater differences to the 2005 samples than did the samples from 2003.

**Conclusions:**
The ability of rep-PCR to distinguish staphylococcal isolates at the clonal level indicates that it can be a useful tool for tracking nosocomial and community-acquired MRSA infections. By using this system, clinical laboratories can now perform real-time source tracking, which may be useful for intervention in outbreak situations, reducing healthcare costs, reducing length of hospital stays, and the spread of antibiotic-resistant organisms.

The ease of use, automation, and standardization of rep-PCR coupled with a format that allows storage of the data in a database as digitalized images of the fingerprint-patterns allows for comparison against the stored database for identification and typing purposes. The automation also reduces interpretation errors and the time to result.

Our findings also provide good support to the hypothesis that the hospital has an "endemic strain" of MRSA.

Finally, with the increased number of CA-MRSA isolates entering the hospital environment it will become even more important to determine the source of these infections and be able to prevent spread of infection with CA-MRSA in the hospital environment.

**References:**